

MYCOLOGIA

VOL. XVI

SEPTEMBER, 1924

No. 5

SUMMARY OF INVESTIGATIONS ON CLOVER RUSTS¹

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(WITH PLATE 14)

For more than thirty years, there has been some uncertainty regarding the species of rusts belonging to the genus *Uromyces* parasitizing our common clovers, white (*Trifolium repens* L.), red (*Trifolium pratense* L.), alsike (*Trifolium hybridum* L.) and zigzag (*Trifolium medium* L.). Mycologists and pathologists have generally accepted the fact that the rust on white clover is a species distinct from the rust on red clover but have been more undecided as to the identity of the species of *Uromyces* on alsike and zigzag clovers. Some maintain that the species on white clover also parasitizes alsike and zigzag, while others believe that the rust on red clover parasitizes alsike and zigzag. The existence of pycnial and aecial stages of *Uromyces* on red; zigzag and alsike clovers has also been in doubt. The investigations described below were undertaken by the writer in an effort to answer the questions:

1. Is there one or are there several species of *Uromyces* on white, red, alsike and zigzag clovers?
2. Do the pycnial and aecial stages of *Uromyces* appear on red, alsike and zigzag clovers?
3. What morphological differences are to be found in the rusts on white, red, alsike and zigzag clovers?

¹ After this article was sent to MYCOLOGIA, the author noted in Bot. Abstr. 13: 272, Mr. 1924, under No. 1834 that Kobel has presented a preliminary note on the specialization and morphology of three of these rusts.

[MYCOLOGIA for July (16: 135-202) was issued August 16, 1924]

4. Will inoculations within the host species and reciprocal inoculations with the different spore forms from each host confirm or disprove the opinion regarding the species of *Uromyces* on these four species of *Trifolium*?

HISTORICAL

Howell (4)¹ observed aecia on red clover plants removed from the field and planted in the greenhouse. She believed the aecial stage existed throughout the winter as mycelium and pycnia but she did not definitely state that aecia were observed on red clover outdoors. She believed there is no doubt that the aecial stage on red clover is identical with that on white clover. Inoculations were made with aeciospores and urediniospores but the results were confusing due to the fact that the hosts were not named. Also, the teliospores were not germinated successfully. But she came to the conclusion that the rust on white and red clovers is one species.

Liro (6) showed by his observations and inoculations that the rust on white clover is autoecious, a eu-type and a distinct species from that on red clover, basing his conclusions biologically on inoculations and morphologically on a variation in the number of germ pores in the urediniospores. He made no mention, however, of pycnial and aecial stages on red, alsike and zigzag clovers.

Kern (5) directed the attention of American mycologists to Liro's discovery of a white clover rust, *Uromyces Trifolii-repentis* (Cast.) Liro, and a red clover rust, *Uromyces Trifolii* (Hedw. f.) Lév. He stated that the aecial stage found on some other clovers belongs with the uredinial and telial stages of white clover rust as the aecial stage on alsike clover reported by Rostrup in Europe is connected with the uredinial and telial stage of *Uromyces* on white clover. Kern also suggested changes in nomenclature, the discussion of which will be omitted (Table IV).

It is to be noted that considerable uncertainty has existed concerning the spore forms, life histories and species of *Uromyces* on red, alsike and zigzag clovers.

Davis and Johnson (A. A. A. S. 1915) reported the aecial stage

¹ Numbers in parentheses refer to literature cited.

of a rust on red clover; Davis (2) described the aecial stage of alsike clover rust and (3) reported all spore forms of red clover rust on zigzag clover. These reports and additional studies have shown that an autoecious, eu-type rust parasitizes each of the following clovers: *T. repens*, *T. pratense* and *T. hybridum*.

METHODS

Field observations and collections of *Uromyces* on species of *Trifolium* were made in nine states extending over a period of eight years. Plants infected with the rust were marked and kept under careful observation throughout different seasons. Different clover plants were grown from sterilized and unsterilized clover seeds in the greenhouse and outdoors. Clover plants with stems and leaves infected with *Uromyces* were removed from the field and planted in the greenhouse during the winter and early spring.

Permanent slides were made from leaves of the different hosts bearing all spore forms. Best results were obtained by employing Fleming's fixative and by staining with Fleming's triple stain employing safranin, gentian-violet, and orange G.

Inoculations were generally made by spraying clover leaves with a composite mixture of viable, germinable spores and distilled water but better results were obtained by allowing aeciospores to drop from the aecia onto the wet clover leaflets.

In all spore measurements, special care was taken to obtain "fair spore samples" which were mounted in distilled water while the spores were fresh. Before germ pores were counted, the urediniospores were submerged for several weeks, in a solution of lacto-phenol containing crystals of Grüber's säuer grün.

SPORE GERMINATION

The germination of teliospores was given special attention because, as has been stated, the conditions for their germination were unknown. Leaves of white, red and alsike clovers bearing telia were stored in corked bottles lined with damp filter paper and incubated at different temperatures, 6°, 12°, 18°, and 20° C. During each week, the germination of teliospores from these and the outdoor materials was tested. For determining the most favorable

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temperature for the germination of aeciospores, urediniospores and teliospores from the different hosts, portions of composite spore samples were placed in distilled water on slides within Petri dishes which were placed in the different compartments of an Altman incubator and in electric ovens.

For one year, weekly spore germination tests were made of outdoor spore materials from white, red and alsike clovers and resulted as follows:

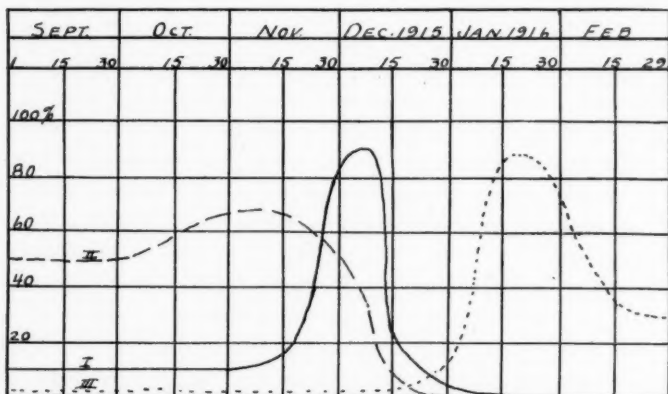


FIG. 1. Curves showing the effect of fall and winter temperatures on the germination of aeciospores, urediniospores and teliospores of *Uromyces Trifolii-repentis* parasitizing *Trifolium repens* (Madison, Wisconsin 1915-1916). I. Aeciospores; II. Urediniospores; III. Teliospores.

1. Aeciospores from white clover germinated about ten per cent in dry weather but the percentage of germination increased after fall rains until December 7 when the temperature was -14°C . Thereafter, the percentage of spore germination decreased decidedly but aecial material was collected during the first part of the open winter until the severe weather in January. Aecia have not survived the northern winters from 1915 to 1924; however, they have been observed in the field during every month of the year except January, February and March.

2. Viable aeciospores have been collected from red, zigzag and alsike clovers in the field during the months of April, May, June and July only. During these months, each properly conducted

germination test showed a good percentage of germinated aecio spores.

3. Urediniospores from white clover germinated about fifty per cent during the dry summer but germination increased during the cool fall until a sudden drop in temperature to -14°C . on December 7. Urediniospores failed to germinate in February and have not remained viable in the northern regions where the work has been in progress for the past 8 years. The results for the seasonal germination tests of the urediniospores from red, zigzag and alsike clovers followed closely those already described for white clover.

4. Teliospores from white clover began to germinate during the latter part of December but generally germinated best during the first week in February. A decided drop in the percentage of germination was noted during February, 1916. But during the winter of 1916-1917, the best germination was obtained during the latter part of March. The seasonal variation in the germination of teliospores was greater than for any other spore form tested. A few germination tests showed germinated teliospores during each month of the year but the percentage was sometimes negli-

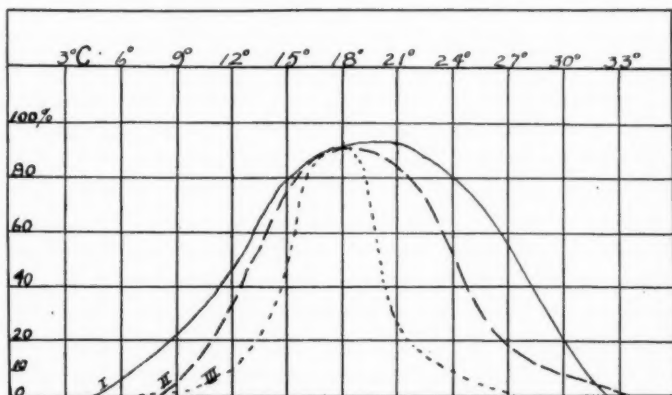


FIG. 2. Curves showing the minimum, optimum and maximum temperatures for the germination of aeciospores, urediniospores and teliospores of *Uromyces Trifolii-repentis* parasitizing *Trifolium repens*. I. Aeciospores; II. Urediniospores; III. Teliospores.

gible. This fact may account for the appearance of aecia throughout the growing season.

5. Teliospores from red clover germinated 10 per cent on January 15; 15 per cent on February 28; 30 per cent, March 15; 50 per cent, April 15; 70 per cent, April 30; 10 per cent, May 15 and 0 per cent, June 1, 1916. Thus the germination of teliospores began in January, was most favorable in March and April but had ceased during the following summer.

Seasonable laboratory spore germination tests of teliospores from zigzag and alsike clovers gave results similar to those described for red clover.

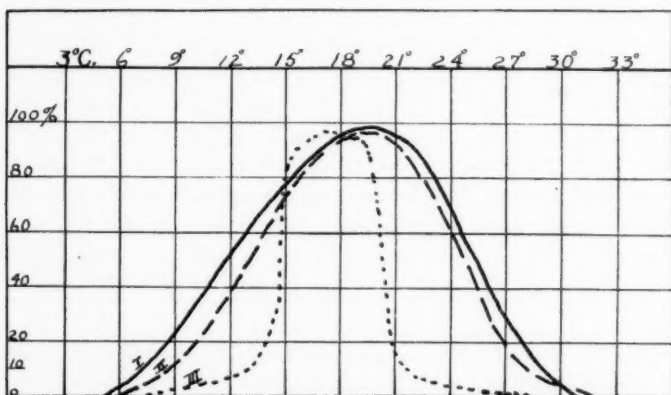


FIG. 3. Curves showing the minimum, optimum and maximum temperatures for the germination of aeciospores, urediniospores and teliospores of *Uromyces Trifolii* parasitizing *Trifolium pratense*. I. Aeciospores; II. Urediniospores; III. Teliospores.

TEMPERATURES: Aeciospores, urediniospores and after-ripened teliospores of *Uromyces* on white, red and alsike clovers were set to germinate under controlled temperatures. Aeciospores from white, red and alsike clovers germinated at a minimum temperature of 6° C.; optimum, 18–20° C.; maximum, about 30° C. This maximum is 4° C. above that reported by Howell. The urediniospores from each of the three clover hosts germinated as follows: minimum, 8° C.; optimum, 19° C. and a maximum slightly above 30° C. Howell placed the maximum at 25° C. The teliospores

from all three clovers mentioned above germinated at a minimum of 7° C.; optimum, 17° C. and a maximum slightly under 30° C. For ascertaining the best temperature for sporidial formation, two trials with teliospores from red and alsike clovers gave the following results: minimum, 10° C.; optimum, 17° C. and a maximum of 21° C. Thus if the formation of sporidia is to be taken into consideration, the range of temperatures for the germination of teliospores is much narrower than that already stated above.

INOCULATIONS

Liro (6) failed to obtain infection on alsike clover inoculated with aeciospores, urediniospores and sporidia from white clover. He did not attempt reciprocal inoculations. As previously mentioned, Liro showed by his inoculations the existence of two rust species, one on white and one on red clover.

The author has made over 100 inoculations with different spore forms from white, red, alsike and zigzag clovers to determine the biological characters and the status of existing species. White, red, zigzag and alsike clovers were each inoculated with sporidia, aeciospores and urediniospores from white, red, zigzag and alsike clovers. The results follow:

1. Aeciospores infected where inoculations were made within the host species, cross-inoculations being unsuccessful on white, red and alsike clovers. Cross-inoculations and inoculations within the species produced infection in red and zigzag clovers. These infections produced the characteristic urediniospores in each case.

2. Urediniospores likewise infected where inoculations were made within the host species, cross-inoculations being unsuccessful on white, red and alsike clovers. Cross-inoculations and inoculations within the species were successful on red and zigzag clovers. Urediniospores produced the characteristic uredinia and old plants showed the presence of characteristic teliospores which were sometimes in the same sorus with urediniospores.

3. Teliospores produced sporidia which infected where inoculations were made within the host species, cross-inoculations being unsuccessful on white, red and alsike clovers. Sporidial infection resulted in aecia characteristic of the host species.

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4. Thus, biologically, there is a separate species of *Uromyces* on each of these three clovers, white, red and alsike. The species of rust on zigzag clover is the same as that on red clover.

5. Two inoculations of *Trifolium incarnatum* L., crimson clover, were made with aeciospores, urediniospores and sporidia on germinated teliospores from *T. repens*, *T. pratense* and *T. hybridum* but no infection was obtained. Repeated injury to some of the plants prevents a definite conclusion at this time.

MORPHOLOGICAL STRUCTURES

Since the inoculations showed there is one biologic species of rust on each of the three clovers—white, red and alsike—observations and measurements were now made to detect variations in morphological structures and the limits of variability for each species of rust. For this purpose, free-hand sections and mounts were made of well developed rusted materials from each of the three hosts bearing all stages of the rusts. Permanent mounts were also prepared by treating each stage of the rust in each host with the same kind of killing solution and employing the same staining process.

TABLE I

COMPARATIVE MEASUREMENTS FOR THE DIFFERENT PARTS OF PYCNIA ON SLIDES PREPARED FROM INFECTED CLOVER LEAVES COLLECTED IN THE FIELD

All measurements are in microns and data are averages from 20 measurements.

Hosts	Diameter Ostiole	Hymenial				
		Height	Width	Surface	Paraphyses	Pycnospores
<i>T. repens</i> . . .	17	150	120	35	(1) 2-4 x 40-65 (2) 2.5 x 40	1.7-2.4 x 2.4-3.6 2 x 3
<i>T. pratense</i> . .	25	100	100	25	2-5 x 34-40 3 x 40	2-3.5 x 3.5-5 3 x 4
<i>T. hybridum</i> .	26	119	119	30	2-4 x 40-85 3 x 65	2-3 x 4-5 3 x 4

1. Limits.

2. Standards which include over 65 per cent of the specimens measured.

PYCNIA: In comparing the pycnia of the rust on alsike clover with those on white clover, these facts were noted: (See Table I; also Plate 14, A, E.)

1. The pycnia in alsike clover are shorter, not extending so deeply into the host tissues.
2. The ostiole is of greater diameter.
3. The hymenial surface is somewhat shallower.
4. The paraphyses are longer than those from pycnia in white clover.
5. The pycnospores are slightly larger.
6. The sizes of the pycnia, pycnospores and diameter of the ostioles compare more favorably with those of the rust on red clover than those of the rust on white clover.

TABLE II

COMPARATIVE MEASUREMENTS FOR THE DIFFERENT PARTS OF AECIA ON
SLIDES PREPARED FROM INFECTED CLOVER LEAVES
COLLECTED IN THE FIELD

All measurements are in microns and data are averages from 20 measurements. Op. = Aecia opened; Cl. = Aecia closed.

Hosts	Heights	Widths	Peridial Cells					Openings
			Walls		Quotients	Lumina		
			Outer	Inner		Lengths	Widths	
<i>T. repens</i>	224	256	2.4-4.8 Op. 3.2 Cl. 4.5	1-2 1.2 1.5	3	14-20	8-12	310
<i>T. pratense</i>	168	192	2.4-4.8 Op. 3.6 Cl. 4.0	1-2.5 1.6 2.0	2	12-16	8-10	224
<i>T. hybridum</i> . . .	208	208	2-5 Op. 3.2 Cl. 4.0	1-1.6 1.2 1.6	2.5	10-14	8-12	140

AECIA: (Table II; also Plate 14, Figs. A, B, D, G, H.). Aecia on alsike clover are correspondingly smaller than those in white clover, extend deeper in the host tissues, but about the same width as those in red clover.

Measurements of peridial cells varied considerably. When the aecia in all these three hosts were closed, the outer and the inner peridial walls were thicker than when opened. Also the peridial walls were much thicker in sections of fresh aecia made with a freezing microtome and mounted in lacto-phenol than in prepared slides. On account of this variation, two measurements from pre-

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pared slides only are reported in Table II; namely, in opened aecia and in closed aecia. The measurements of peridial walls in the closed aecia are considered the more accurate for there were fewer variations.

The outer walls of the peridial cells in closed aecia from alsike clover are correspondingly thinner than those in white clover but the inner peridial walls are of about the same thickness. In red clover, the outer peridial walls are of about the same thickness as those in alsike clover while the inner walls are somewhat thicker.

The lumina of the peridial cells in alsike clover are shorter than those in the other two clovers; the widths in all three clovers being comparatively the same.

Measurements of opened aecia on leaves showed that the peridial cells in alsike clover do not normally open vertically or become revolute like those in white and red clovers but remain slightly incurved. Also, the aecia in alsike clover are wider, shorter, the outer peridial walls thinner, and the peridial cell lumina shorter than those in white clover.

The spores from aecia in white clover have been assigned different measurements by different mycologists (See Table III—also Plate 14, *B*). The measurements reported by the writer were made from emitted, fresh aeciospores mounted in water. No great differentiation in size was noted in the spores from aecia on white, red and alsike clovers. Aeciospores from alsike clover are slightly smaller than those from the other three clovers.

UREDINIOSPORES: (Table III; also, Plate 14, *C, F*.) The germ-pores in the urediniospores of the rust on white and alsike clovers are similar in number, 2 to 4, and similarly placed, equatorial. The urediniospores from the rust on red clover possess 5 to 7 germ-pores which are scattered. Thus there is a distinctive morphological difference, regarding the number of germ-pores, between the urediniospores from the rust on white clover and on red clover. However, it is to be noted in Table III that Arthur and Sydow give nearly the same measurements for urediniospores from these two hosts.

Examination of urediniospores from the rusts on white, red and alsike clovers verified the morphological differences as previously

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stated. However, the urediniospores from alsike are slightly longer than those from white clover.

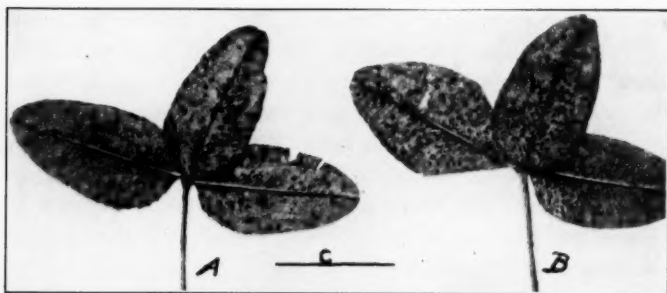


FIG. 4. Leaves of *Trifolium hybridum* bearing uredinia and telia of *Uromyces Hybridi*. A. Leaflets bearing mostly uredinia. B. Leaflets bearing mostly telia. C. Length of a leaflet.

TELIOPORES: (Table III; also, Plate 14, C, F.) No constant, definite morphological differences were noted in the teliospores from the rust on white, red and alsike clovers. The measurements were practically the same save some especially long teliospores were found in samples from alsike clover and, in some samples, the teliospores were of a darker color.

PROMYCELIA AND SPORIDIA: (Table III.) Promycelia from the germinating teliospores removed from white, red and alsike clovers averaged $7-8\mu$ in diameter, varied from 48 to 170μ in length and contained 4 to 5 cells. The sterigmata were 2 to 3μ in diameter, generally widest near the promycelium and from 4 to 7μ long. The narrowest and longest sterigmata were found on the promycelium from teliospores of red clover rust. The sporidia averaged 7μ in diameter, 10 to 14μ in length and germinated at once. The average size of sporidia on promycelia from germinating teliospores of white clover were smallest while those of alsike clover averaged the largest, 14μ long; otherwise, the germinated teliospores and their parts were similar in size and structure for the rusts on all *Trifolium* hosts examined.

TAXONOMY

Howell classified the rusts on white and red clovers as *Uromyces Trifolii* (Alb. and Schw.) Wint. while Liro showed there are two species on these clovers, *Uromyces Trifolii-repentis* (Cast.) Liro. on white clover and *Uromyces Trifolii* (Hedw. f.) Lév. on red clover.

TABLE IV

GENERA AND SPECIES TO WHICH DIFFERENT AUTHORS HAVE ASSIGNED THE RUSTS ON FOUR DIFFERENT *Trifolium* SPECIES

Authorities	<i>T. repens</i>	<i>T. hybridum</i>	<i>T. pratense</i>	<i>T. medium</i>
Liro.	<i>Uromyces Trifolii-repentis</i>	—	<i>U. Trifolii</i>	—
Sydow. . . .	<i>Uromyces Trifolii-repentis</i>	<i>U. Trifolii</i>	<i>U. Trifolii</i>	<i>U. Trifolii</i>
Kern.	<i>Uromyces Trifolii</i>	<i>U. Trifolii</i>	<i>U. fallens</i>	<i>U. fallens</i>
Arthur. . . .	<i>Nigredo Trifolii</i>	<i>N. Trifolii</i>	<i>N. fallens</i>	<i>N. fallens</i>
Davis.	<i>Uromyces Trifolii-repentis</i>	<i>U. Hybridi</i>	<i>U. Trifolii</i>	<i>U. Trifolii</i>

From Table IV, it is to be noted that various authors assign the rust on red and zigzag clovers to the same species but there is a disagreement regarding the species of rust on alsike clover. Kern and Arthur classify the species of rust on alsike with that on white clover while Sydow lists it with the rust on red clover.

In general, mycologists have referred the rust on white and alsike clovers to the same species because, as has been stated, the urediniospores from each *Trifolium* host possess 2 to 4 germ pores while those from red clover possess 5 to 7 germ pores. The writer believes too much weight has been given to the number of germ pores in urediniospores for establishing species in the clover rusts. Should mycologists assign the rust on legumes, *Uromyces Pisi* (Pers.) Wint. to *Uromyces Trifolii* (Hedw. f.) Lév., the red clover rust, because some urediniospores of each possess 5 germ pores? Liro was first to point out these differences in germ pores for classifying the clover rusts and his classification was adopted by Sydow (Fig. 7). However, Liro was unable to inoculate alsike clover with aeciospores and urediniospores from *T. pratense*.

Thus the classification of the rust on alsike clover has remained unsolved because the aecial stage was unknown and sufficient inoculations were not made to determine its hosts.

As has been stated, the aecial stage of the rust on alsike clover has been observed and inoculations show it to be a separate eucytype rust, *Uromyces Hybridi* sp. nov. A description of the organism follows.

Uromyces Hybridi sp. nov.

PYCNIAL STAGE: Pycnia amphigenous, within inflated, yellow areas often grouped on veins of leaf blades and on petioles, light yellow changing to purple then grey, globose, width $119\ \mu$, height $119\ \mu$; ostiole $26\ \mu$ in diameter; hymenial surface $30\ \mu$; paraphyses $3 \times 65\ \mu$; pycnosporos $3-4\ \mu$.

AECIAL STAGE:¹ Aecia amphigenous, in puffed yellow areas on blades, petioles and stipules, sometimes scattered, surrounding pycnia; diameter $208\ \mu$, height $208\ \mu$; peridium yellow, margin slightly incurved, coarsely erose; peridial cells overlapping, lumen $8-12\ \mu$ wide by $10-14\ \mu$ long, outer wall $2-5\ \mu$ thick, average $4\ \mu$ and transversely striate, inner wall $1-1.6\ \mu$ thick, average $1.6\ \mu$ and verrucose; aeciospores globose to ellipsoid-angular, $17-20 \times 21-24\ \mu$; wall yellow to colorless, $1\ \mu$, verrucose.

UREDINIAL STAGE: Uredinia mostly hypophyllous, scattered, elongate to roundish, $0.08-0.5\ \text{mm.}$ wide by $0.12-0.6\ \text{mm.}$ long, somewhat covered by the conspicuously ruptured epidermis, pulverulent, cinnamon-brown; urediniospores ellipsoidal to globose, $20-22 \times 22-24\ \mu$; wall cinnamon to hazel; germ pores $2-4$, equatorial; hilum $5.5\ \mu$.

TELIAL STAGE: Telia mostly hypophyllous, scattered, attenuate-elongate to circular, hazel, $0.14-0.4\ \text{mm.}$ wide by $0.17-0.8\ \text{mm.}$ long, epidermis conspicuous and persistent; teliospores globose, ellipsoidal or obovate-ellipsoidal, $17-20 \times 24-28\ \mu$; wall smooth, clay or ochraceous-tawny, $1.5-2\ \mu$, germ pore covered with a hyaline papilla; pedicel clay-colored to colorless, $5\ \mu$ wide by $6.4\ \mu$ long. Promycelia $3-4$ septate, hyaline, $6.8-8\ \mu$ by $48-170\ \mu$; sterigmata $3 \times 4\ \mu$; sporidia hyaline, smooth, obovate to globose, $7 \times 14\ \mu$.

Description of the pycnial and aecial stages of *Uromyces Trilolii* (Hedw. f.) Lév. on *Trifolium pratense* L.

PYCNIAL STAGE: Pycnia amphigenous, grouped, light yellow turning reddish then grey, globose, $100\ \mu$ wide, length $100\ \mu$; hymenial surface $25\ \mu$, paraphyses $3 \times 40\ \mu$; ostiole $25\ \mu$ in diameter; pycnosporos $3 \times 4\ \mu$.

AECIAL STAGE: Aecia amphigenous, grouped with the pycnia in

¹ Davis, W. H. The aecial stage of alsike clover rust. Proc. Iowa Acad. of Sci. 24: 461-472. 1917.

elongated areas on blades, petioles and stipules; color at first golden, changing to gray, $192\ \mu$ in diameter, $168\ \mu$ high; peridium yellow-white, margin recurved or sometimes revolute, lacerate; peridial cells overlapping; lumen $8\text{--}10\ \mu$ wide by $12\text{--}16\ \mu$ long; outer peridial wall limits $2.4\text{--}4.8\ \mu$ thick, average $4\ \mu$ and transversely striate; inner wall limits $1\text{--}2.5\ \mu$ thick, average $2\ \mu$, slightly verrucose; aeciospores globose to ellipsoid or angular, $17\text{--}22 \times 20\text{--}26\ \mu$; wall light yellow to hyaline, $1\ \mu$ thick, minutely verrucose.

SUMMARY

The pycnial and aecial stages of the rust on alsike, red and zigzag clovers have been produced by artificial inoculations, collected in the field and described.

The following morphological differences between the rust on alsike and the rust on white clover were observed:

1. Pycnia from alsike clover are:
 - a. Shorter.
 - b. The ostioles of greater diameter.
 - c. The hymenial surfaces shallower.
 - d. Paraphyses longer.
 - e. Pycnosporos slightly larger.
2. Aecia from alsike clover are:
 - a. Wider and shorter.
 - b. Outer peridial walls thinner.
 - c. Lumina in peridial cells are shorter.
 - d. Peridial cells of the opened aecium are slightly incurved rather than revolute.
 - e. Aeciosporos slightly smaller.
 - f. The aecia generally appear on large yellow, puffed areas surrounding pycnia.
3. Uredinial and telial stages from alsike clover:
 - a. Urediniosporos are slightly longer.
 - b. In general, the differences observed in this stage were very slight.

The inoculations with the different spore forms from alsike clover showed:

- a. Sporidia from germinated teliosporos produced pycnia and aecia on alsike clover but failed to infect white, red and zigzag clovers.
 - b. Aeciosporos produce the characteristic urediniosporos on alsike clover but failed to infect the other 3 clovers.
 - c. Urediniosporos produce the characteristic teliosporos.
 - d. Sporidia, aeciosporos and urediniosporos from white, red and zigzag clovers do not infect alsike clover.
 - e. The rust on alsike clover is autoecious, a eu-type, and not the same biologic species which infects white clover.
4. Inoculations with spores from red clover showed:

- a. Sporidia, aeciospores and urediniospores infect red and zigzag clovers producing in turn the characteristic spore forms. The spores did not infect white and alsike clovers.
- b. Sporidia, aeciospores and urediniospores from alsike and white clovers failed to infect red clover.

Inoculations with spores from white clover:

- a. These inoculations verified Liro's results and showed the rust on white clover is a different species biologically from that on alsike, red and zigzag clovers; an autoecious, eu-type rust.

Tabulated summary giving the common name for each rust, scientific classification for each organism and name of the host.

Common Name of the Disease	Scientific Classification of the Organisms	Hosts
1. White clover rust....	<i>Uromyces Trifolii-repentis</i> (Cast.) Liro.	White clover, <i>Trifolium repens</i> L.
2. Red clover rust.....	<i>Uromyces Trifolii</i> (Hedw. f.) Lév.	Red clover, <i>Trifolium pratense</i> L. and Zigzag clover, <i>Trifolium medium</i> L.
3. Alsike clover rust....	<i>Uromyces Hybridi</i>	<i>Trifolium hybridum</i> L.

Therefore, there is a separate species of *Uromyces* with all spore forms on each of these clovers, white, red and alsike. The rust on alsike is not the same as on white clover but a separate species, *Uromyces Hybridi*.

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EXPLANATION OF PLATE 14

Photographs and photomicrographs showing various stages of *Uromyces Hybridi* on *Trifolium hybridum* (A-F) and *Uromyces Trifolii* on *Trifolium pratense* (G-H). B-F were photographed from prepared slides.

Figure A. Aecia and pycnia on the under side of two young alsike clover leaves.

1. Pycnia on a leaflet.
2. Aecia in swollen areas on the midrib.
3. Aecia on a petiole.

Figure B. Section of an aecium from alsike clover.

1. Aeciospore showing the verrucose wall.
2. Outer peridial walls transversely striated.
3. Verrucose inner walls of the peridial cells.

Figure C. Section of an alsike clover leaf showing a telium and uredinium on the lower surface.

1. Lower epidermis of the host.
2. Teliospore with pedicel.
3. Urediniospore.

Figure D. Peridial cells in an aecium from alsike clover.

- 1-2. Transversely striated outer walls of the peridium.
3. Inner verrucose wall of the peridium.

Figure E. Section of a pycnium in an alsike clover leaf.

1. Long paraphyses extending through the ostiole at 2.

Figure F. Urediniospores, from alsike clover, mounted in lacto-phenol containing sauer grün.

- 1-3. Urediniospore with germ pores.
- 4-5. Teliospores; each bearing a hyaline papilla over the germ pore.
- 6-7. Echinulated surfaces of urediniospores.

Figure G. Aecia of *Uromyces Trifolii* on the midrib of a leaflet of *Trifolium pratense*.

Figure H. Aecia on a leaflet of *Trifolium pratense*.

THE MORPHOLOGICAL DEVELOPMENT OF *ASTEROSPORIUM HOFFMANNI*¹

W. ANDREW ARCHER

(WITH PLATES 15 AND 16)

The genus *Asterosporium* and the species *Asterosporium Hoffmanni* were established by Kunze (1) in 1819 from the type species *Stilbospora asterospora* Hoff. Many figures and articles have appeared in various books and journals regarding the nature of this fungus but no one has given an accurate account of spore formation. Fresenius (3) gave a few sketches of the conidia and a short account of the fungus. He states: "Ich habe auf der Tafel, (V. Fig. 13) auch einige noch einfachere jugendliche Sporen abgebildet; das erste Entstehen derselben an den Fäden aber ist bis jetzt von mir nicht beobachtet." Then he quotes from a Herr Reiss, without giving any reference: "auf einem stratum proliferum, das aus hellen schlaffen, einfachen dicht gedrängten Fäden besteht. Diese entwickeln an ihrem oberen Ende ein Bläschen, das bald rundliche Vorsprünge treibt und durch deren Verlängerung zur Spore auswächst." Later Zopf (4, p. 36) refers to this work of Fresenius and makes the statement: "Sehr eigenthümliche Gestaltung zeigen nach Fresenius die mehrzelligen Conidien von *Asterosporium Hoffmanni*. Sie sind nämlich aus 4 kegeligen, in Centrum zusammenstossenden, mehrzelligen Strahlen gebildet. Ueber die Entstehungsweise dieser Conidienform fehlen noch Untersuchungen."

As far as could be determined, this fungus has not been cultured before. It grows rather readily on Leonian's synthetic agar (5). Single spore isolations were made, following the technique for the spray method as given by Kauffman (6). The cultures were made in small capsules (glass dishes of about 25 cc. capacity with loose-fitting lids). The temperature was that of

¹ Papers from the Department of Botany of the University of Michigan, No. 211.

the laboratory. At various stages of development, material was imbedded for microtome sections to study the morphology of the fruit body; while the development and structure of the conidia were determined by teasing out the contents of young fruit bodies and by examination of sectioned material. For staining sections a combination of Delafield's haematoxylin and analin safranin was used.

The collection which formed the basis for these studies was made in April 1923, along the river near Ann Arbor on fallen branches of *Fagus grandifolia* Ehrh.

It requires at least one to four days for conidia to germinate when sprayed on Leonian's agar; many of them germinating even after ten days. A single germ tube arises from the tip of each arm (Pl. 15, fig. 6), but the germination of some of the arms may be much delayed or else may fail to take place at all. These last two facts would indicate that each arm functions physiologically as an individual spore; of this more will be said later. The germ tubes elongate slowly for a few days, laying down an occasional wall (Pl. 15, fig. 7); then they begin to branch rather sparingly at the ends (Pl. 15, fig. 8). Soon the mycelium begins to be evident to the naked eye. It develops rather slowly and scantily, rarely ever covering the entire available surface of the medium. The aërial growth is scanty, confined to the center of the colony and consists of straggly wefts of long, whitish hyphae. Submerged in the medium are formed globose masses of mycelium; these are outlined by the blackened tips of their hyphae. From above, the surface of the culture appears as a whitish mat tinged with brown.

The young fruit bodies are just perceptible to the naked eye after ten days, or even in less time in some cultures. They gradually increase in size forming irregularly globose, superficial bodies that are easily detached from the mycelium. When about half-grown they have a striking resemblance to young colonies of *Nostoc* due to their greenish translucence. Even after mounting in water on a slide they still show this resemblance in that they have a gelatinous tenacity making it difficult to separate them into pieces small enough for microscopic examination. The fruit

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bodies are fully mature after about four weeks; when they appear as clustered, black heaps on the surface of the medium.

The primordium of the fruit body arises from a knarl of hyphae after the fashion designated as symphogenetic by DeBary (12, p. 247). Such a knarl (Pl. 15, fig. 1) by continued branching of the hyphae increases in size and forms a compacted interior, *i.e.*, the plectenchymatous primordium (Pl. 15, fig. 2). At this stage there is a differentiation of an outer layer made up of ten to fifteen filaments of coarsely interwoven hyphae (this number is only approximate, for as a matter of fact, this wall can vary a great deal in thickness). In mature fruit bodies it is still evident but it is no longer coarsely interwoven; it is more tightly stretched and the individual strands are more or less fused together (Pl. 15, fig. 3).

The first conidia start forming as soon as, or even before, the primordium is well defined, appearing, as a rule, near the center of it. The primordial tissue that initiates conidial formation is stained deeply by the safranin, in contrast with the yet undifferentiated portions. This indicates some constitutional change, probably chemical, in the tissues. There is no definite hymenial layer, for the swollen primary cells of the conidia,² each with its attached conidiophore, can be seen lying in all directions, intermingled with the woven hyphal strands of the primordium (Pl. 15, figs. 2 + 4). The early signs of a cavity become evident when these first conidia begin to mature and use up the primordial tissues (Pl. 16, fig. 1). At this time the entire primordium interior is dotted with the initials of young conidia and as spore production proceeds the tissue of the primordium disappears, signifying a lysigenetic action as interpreted by Dodge (9, p. 745) and originally defined by DeBary (12). Intermixed with these conidia can be found for a time various, irregular fragments of unused primordial tissue. Beyond the periphery of the cavity young conidia are imbedded at various levels in the rapidly disappearing available tissue. Finally this is used up and there remains only the outer layer of hyphae that forms the wall and at this time the fruit

² The spores of this fungus are termed conidia because of their position and mode of origin, but they could be termed pycnidiospores with reference to the structure in which they are borne.

body can be said to be mature (Pl. 15, fig. 3). All signs of the disintegrated primordial tissue have disappeared. In old cultures the upper portion of the pycnidial wall dries, ruptures, and falls off leaving an irregular, saucer-shaped lower portion heaped with a powdery mass of conidia.

The young stages of the development of the fungus on its natural substratum have not been observed but there is every reason to believe that they are similar to those just described. Indeed, careful observation of the mature stage on the natural substratum revealed the presence of an outer surrounding wall of hyphae much as it appeared in the cultures (Pl. 15, fig. 5).

A glance at Pl. 15, figs. 2-3 will show that the normal tendency of the fruit body is to be globose. This is due, no doubt, at first to the turgor of the internal compactness of hyphae and later to the great number of conidia. The fruit body in the natural substratum is convex or cushion-shaped merely because it has been confined by the periderm of the bark. In fact it seems clear that it is this tendency of the fruit body to become globose that causes the rupture of the restraining periderm.

The conidiophore is merely a slightly differentiated branch from a strand of the woven hyphae in the primordial tissue (Pl. 15, fig. 4; Pl. 16, fig. 2). The conidiophores are somewhat more slender, as a rule, than the hyphae of the primordium from which they arise, and generally have several septa. As the cavity forms, the conidiophores arise more and more from the remaining primordial tissues at the periphery. A few project in various lengths into the cavity, but most of them are obscurely imbedded in the tissue itself. Finally the whole interior of the pycnidium is filled with conidia, the disorganized primordial tissue has disappeared and the only conidiophores remaining are those attached to the inside of the pycnidial wall (Pl. 15, fig. 3). When the conidia first start forming they are tightly surrounded by the hyphae of the primordium (Pl. 15, fig. 4). It is clear, in the case of *Asterosporium*, that space for spore formation is obtained by the production, from the protoplasm of the actively forming conidiophores and conidia, of some enzyme capable of disorganizing and digesting the surrounding tissue. There is evidence of this disorganization in the fact

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that tissues immediately surrounding the conidia stain more deeply with safranin than elsewhere, and by the ragged appearance of the hyphal strands that project into the forming cavity (Pl. 16, fig. 1).

As the free end of the conidiophore enlarges it becomes vacuolated; after a period of swelling the apical portion is cut off from the conidiophore by a septum and thus becomes the primary cell of the conidium; still later a cross wall is formed dividing the primary cell itself into halves (Pl. 16, figs. 3-5). From this point on there is no definite order for the succeeding divisions in the developing conidium. What might be termed the normal order is represented in Pl. 16, figs. 2-9. In the mature pycnidium the four-armed conidium is by far the most common, with the three-armed type constituting the remainder, excepting a very few one-, two-, or five-armed forms. During the development of the four-armed conidium the primary cell first lays down a vertical wall at a slight angle with the plane of the conidiophore, dividing the cell unequally (Pl. 16, fig. 6). Later two other walls form similarly so that a pyramidal segment is left at the center. A diagrammatic side view of such a group of cells is shown in Pl. 16, fig. 13. The three portions cut off in this manner develop into the three respective lower arms by elongation and septation. If the primary cell lays down only one wall, or if two walls are formed with the omission of the third, a three-armed conidium will result. Some of the irregular forms are seen in Pl. 16, figs. 10-12; these, in a mount of young conidia, are by far in the majority but since no such irregularities are found, at least only comparatively rarely, in mature fruit bodies, it is evident that they eventually complete their development. The five-armed form occurs when the basal cell of the top arm produces side arms in much the same way as the primary cell of the normal conidium.

The first stages in the morphologic development of the conidium are best observed from water mounts of fresh material but later, after the spore becomes dark and more complex, it is necessary to use stained sections. A half-mature fruit body teased out on a slide will yield all stages with the exception of fully mature conidia. At the very first the spore initial appears to be entirely hyaline, filled with a clear homogeneous substance, but as soon as

there is any perceptible swelling at the apex, granular protoplasm can be seen pushing up through the conidiophore. As the initial enlarges still further this protoplasm crowds into the spore and a cross wall forms at the base. At each subsequent division of the conidium a portion of the protoplasm is cut off so that in the fully elongated arm the protoplasmic contents have the appearance of a segmented column (Pl. 16, fig. 16). At this point there ensues a differentiation of parts: on the outside there forms a thin exosporium, at first hyaline and soft, but later becoming brown and brittle; at the center of the segment the protoplasm collects in a globose mass, finally surrounded by a definite wall, the endosporium, which is three to four times the thickness of the exosporium; while between the endosporium and exosporium of the lower segments there is a relatively thick, hyaline, faintly staining substance, the exact nature and consistency of which could not be determined. This substance is easily separable, at maturity, from the exosporium and endosporium as seen when the thin exosporium is cracked away leaving it intact or when a cell with its endosporium is crushed out from the conidium. It starts forming near the exosporium and proceeds inward; or in other words, it is deposited by the protoplasmic segments of the young conidium as they round up and become more dense.

At the apex of the arm the exosporium is thickened and marked by a germ pore which is apparently closed by a thin, hyaline, bulged membrane (Pl. 16, fig. 19). In old, dry conidia the flexible nature of this membrane is evident from the fact that it is collapsed inward. The tip of the apical cell projects out into this pore, sometimes touching the hyaline outer membrane.

The cells or protoplasmic units of a conidium are all connected by strands but this is evident only in a state of plasmolysis such as occurs during killing and staining processes (Pl. 16, fig. 17). When the endosporium is fully differentiated it is seen to be tightly pressed against the cross wall. The two lower cells are globose in shape and slightly flattened at the line of contact with the cross walls, while the terminal cell fits tightly into and conforms to the shape of the conical, apical segment (Pl. 16, fig. 18). The two lower cells of an arm, together with the endosporium,

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can be separated out by crushing the conidia. Such a loosening of these parts is reported also for *Helminthosporium* by Drechsler (13, p. 646). In water mounts of conidia, the protoplasm of these cells is densely granular, with rather small, scattered, hyaline areas of various sizes which, in fully matured spores, are condensed into larger droplets (Pl. 16, figs. 20-21). In stained sections it is possible to distinguish several nuclei within the protoplasm of each cell (Pl. 16, fig. 15); while in the hyphae of the primordium that is actively engaged in spore formation, nuclei undergoing division can be made out (Pl. 16, figs. 14 a-c). Their minuteness makes it impossible to see any of the details of mitosis.

The exosporium crushes easily allowing the arms to become broken off and the endosporium units to be released. It must have been an observation of this that lead Kunze (1, p. 227) to consider the conidia to be sporangia. He states: "Diese Masse nun besteht, unter starker Linse besehen, aus dunklen, sternförmigen Sporangien, gewöhnlich mit drey, seltner vier und äusset selten fünf kurzen, etwas stumpfen, geschiedenen Strahlen, welche, unter Wasser gequetscht, sehr kleine, längliche, halbdunkle Sporidien ausleeren. Damit werden die Sporangien heller, die Scheidewände deutlicher."

When germinating, the conidium sends out a germ tube from the hyaline, apical germ pore; the contents of a single arm then passes out into this tube. Details are best obtained by the use of oil immersion and sectioned material of germinated spores. The tip of the terminal cell pushes up until it ruptures the hyaline membrane of the pore and then puts forth an irregular and enlarged tube (Pl. 16, fig. 22); this soon elongates and takes on the uniform diameter and appearance of an ordinary germ tube. The tube then forms cross walls in the usual manner during the next few days, as shown in Pl. 15, fig. 7. About this time, it also produces a heavy peripheral wall that becomes brown with age. In sectioned material a cross wall is revealed just within the apical pore (Pl. 16, fig. 23), evidently formed after germination was complete. The formation of the germ tube causes the terminal cell to lose its turgidity so that it collapses and becomes column-like, appearing to be a continuation of the germ tube. Soon after

the apical cell germinates the contents of the second and third cells pass up through unique structures in the cross walls to join in the formation of the germ tube. Such a structure occurs between any two adjoining cells of an arm (Pl. 16, figs. 22-23) and is formed from the modified and thickened portion of the endosporium or inner wall on either side of the cross wall (Pl. 16, fig. 24). Certainly modification has taken place for the portions concerned stain quite differently from the rest of the walls. The opening through the center of the structure connects the cells, probably by definite cytoplasmic canals, recalling the strand-like connections seen between the immature, plasmolyzed segments (Pl. 16, fig. 18).

Each arm functions physiologically as an individual spore because in sectioned and stained material it is possible to find conidia in which one arm has lost its protoplasmic contents by way of the terminal germ tube while the other arms are still unchanged or else just beginning germination. Furthermore actual openings can be seen between the cells of germinated arms when the mounts are properly manipulated.

Each cell, including the one in the pyramidal segment at the very center of the conidium, is capable of sending out an individual germ tube; although this is to be considered as atypical germination, for reasons that are to be presented. If a quantity of conidia are crushed in a manner to fragment the arms and then sowed on nutrient agar, germ tubes may form from any one or all cells (Pl. 16, fig. 25). In such a fragment the germ tube usually passes through the open, broken end, *i.e.*, the path of least resistance. In such a preparation some conidia that have not been broken into fragments will form lateral tubes through the exosporium, this being true even of the terminal cell (Pl. 16, fig. 26). This again would indicate that the germ tubes are following the path of least resistance, that they are issuing through minute cracks or broken places in the walls. This fact is further emphasized when one prepares two sets of cultures for spore germination, one in which the conidia have been thoroughly broken up and another in which the conidia have been carefully handled. In the latter case practically all the tubes will issue through the regular

germ pore. Despite careful handling however, it is conceivable that a few conidia will be injured due to the fragile nature of the exosporium, so, making due allowance for this fact, it may be stated that normal germination is through the apical germ pore only.

The conidia of *Asterosporium* have a few points in common with those of several species of *Helminthosporium* as described by Drechsler (13, p. 646). In *H. monoceras* there are apical, hyaline germ pores through which normal germination takes place, although if the exosporium is broken the intermediate segments may germinate independently. In *H. teres*, *H. giganteum* and several others there are pit-like places in the cross walls and although Drechsler states that he has been unable to demonstrate definitely whether they really serve as connections between cells, he is inclined to believe that they function in that capacity.

In the discussion of pits in cross walls of spores Zopf (4, p. 366) gives, as examples, *Thielavia basicola* and the teleutospores of *Phragmidium*. He states that, so far as the former is concerned, the pits do not function in germination. He is inclined to believe that such pores, especially in thick-walled spores, serve as a means of cell sap exchange between cells.

GENERAL DISCUSSION

In studying the development of the fruit body of *Asterosporium Hoffmanni* in culture it has been shown that it is a definite globose body with an investing layer of interwoven hyphae; that spore formation originates near the central portion of the primordium and proceeds toward the margins, using up the tissues as it progresses and finally leaving only the enclosing wall of woven hyphae, which persists even in the mature condition. Kunze (1, p. 225) and Saccardo (10, p. 782) mention a "floccoso" stroma, while Diedicke (11, p. 876) describes a "parenchymatischer Basalschicht"; in all three cases the reference, no doubt, being to the lower part of the pycnidial wall in connection with the natural substratum. It is more easily seen at this place since the disruption and drying out of the upper portions usually obscures its continuity.

The method of spore formation in *Asterosporium* is a departure from the usual method found so far in the Sphaeropsidales; since in all described forms the conidiophores are arranged in a parallel manner either around the pycnidial cavity or else on a basal layer of tissue, Dodge (9), DeBary (12, p. 229), and Bauke (7); there being of course the exceptions of such simple forms as *Cicinnobolus* and *Fumago*, described by DeBary (12, pp. 247, 250) and Zopf (4, p. 329). This "hymenium" of parallel conidiophores has been reported regardless of whether the fruit body has a symphogenous or meristogenous origin; Kempton (8, pp. 235, 253) has pointed out that the majority of forms so far described are meristogenous. The symphogenous origin of the primordium in *Asterosporium Hoffmanni* is quite similar to that described for *Guignardia Bidwellii* by Reddick (2), and for the *Diplodia* on *Cornus* by Bauke (7) but here too the cavity is lined with parallel conidiophores. At no time during the process of spore formation in *Asterosporium* is there any semblance of a genuinely parallel arrangement of conidiophores; instead they are scattered and lie in all directions. Also the outline of the cavity is very ragged and irregular as contrasted with the regularity in other forms.

Asterosporium Hoffmanni, according to the usually accepted schemes of classification, belongs to the Melanconiales, the same being true for the genus *Pestalozzia*; yet we have seen that *Asterosporium* has a differentiated outer wall which in advanced maturity breaks open at the top leaving a saucer-shaped lower portion. Kempton (8) has demonstrated the same thing for *Pestalozzia*. These facts indicate the necessity of further knowledge of the early stages of development in many others of the Melanconiales and Sphaeropsidales before final generalizations can be made regarding classification. It is likely with the attainment of such information that many of the present difficulties and discrepancies will be cleared up.

I wish to express my indebtedness to Dr. C. H. Kauffman for his suggestions and guidance in this work.

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EXPLANATIONS OF PLATES

PLATE 15

Fig. 1. Section through knarl of hyphae showing the symphogenous origin of the primordium.

Fig. 2. Section through young fruit body with investing layer of coarsely interwoven hyphae, the plectenchymatous tissue of the interior and the irregular arrangement of spore initials.

Fig. 3. Enlarged segment from 16, indicating the plectenchymatous tissue and the irregular arrangement of conidiophores.

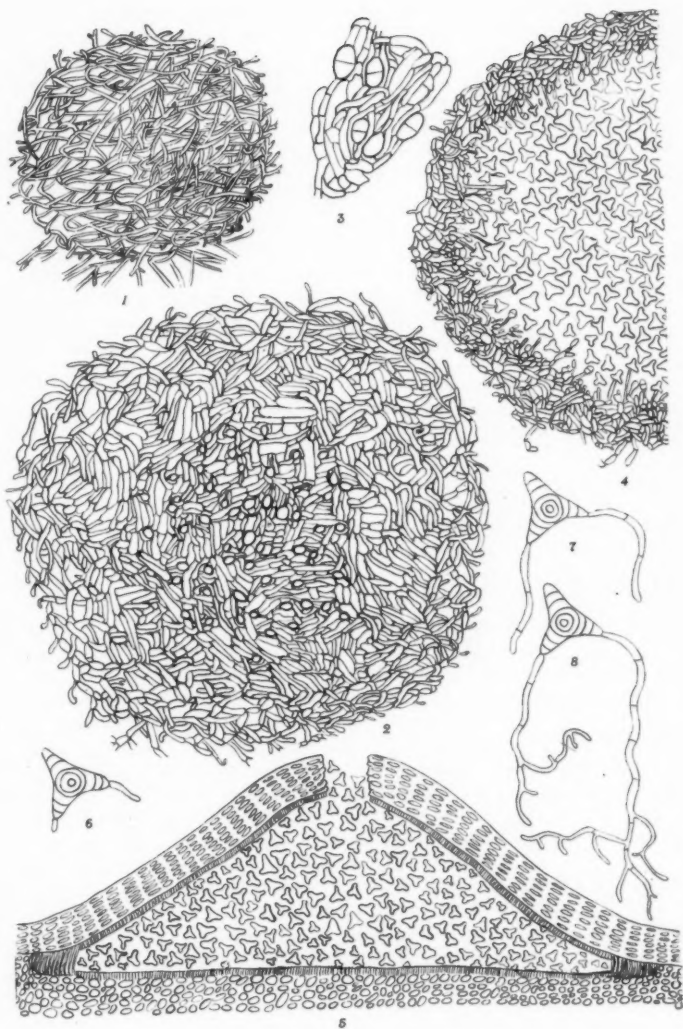
Fig. 4. Portion of section through mature fruit body with large cavity filled with spores. The wall now consists of more or less fused hyphae.

Fig. 5. Mature fruit body on natural substratum. The periderm compresses it into a cushion-shaped form. The surrounding wall of hyphae is present.

Fig. 6. Spore with two arms germinating.

Fig. 7. The germ tubes elongated and segmented by cross walls.

Fig. 8. The germ tubes are branching sparingly at the tips.



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PLATE 16

Fig. 1. Enlarged segment from central portion of fruit body in which cavity formation has started. The lysigenetic action is evident from the space surrounding the conidia and from the disintegrated appearance of the hyphae that project into the cavity.

Fig. 2. Young conidiophore arising from a hypha of the primordium.

Figs. 3-5. Progressive steps in the development of spore initials.

Fig. 6. Formation of the first vertical wall in the primary cell of a young conidium, at an angle with the plane of the conidiophore.

Fig. 7. Formation of second vertical wall of the primary cell.

Fig. 8. Elongation and further septation of arms.

Fig. 9. Mature four-armed conidium.

Figs. 10-12. Types of irregularities seen in immature conidia.

Fig. 13. Diagrammatical view of spore initial indicating the manner in which three segments have been cut off in the primary cell, subsequently to form the three lower arms of the mature conidium, leaving an inverted pyramidal segment at the center.

Fig. 14 a-c. Dividing nuclei in hyphal cells from the meristematic region of the primordium. Triple stain. 1.9 mm. oil-immersion lens.

Fig. 15. Single cell from mature conidium showing nine nuclei in the section. Triple stain. 1.9 mm. oil-immersion lens.

Fig. 16. Fully elongated arm with segmented column of granular protoplasm. External view, in perspective.

Fig. 17. Diagrammatical view of a longitudinal section of an arm of an immature, plasmolyzed conidium, just before the endosporium has fully developed. The protoplasmic units have become contracted during fixing, and are shown to be connected by cytoplasmic strands. The stippled portion represents the wall substance laid down by the protoplasm before the differentiation of the endosporium. (Vide figs. 16 and 18.)

Fig. 18. Longitudinal section of mature arm. The endosporium of the lower cells presses against the cross walls. The terminal cell conforms to the shape of the conical segment enclosing it.

Fig. 19. Detail of apical pore, limited by hyaline, protruding membrane above and by dark exosporium below. The tip of the enclosed cell projects above the edge of the exosporium. The exosporium is thickened near the pore. External view.

Fig. 20. Immature endospore unit, separated out from segment of arm, with scattered droplets of oil-like material.

Fig. 21. Mature endospore unit with the droplets condensed into larger areas.

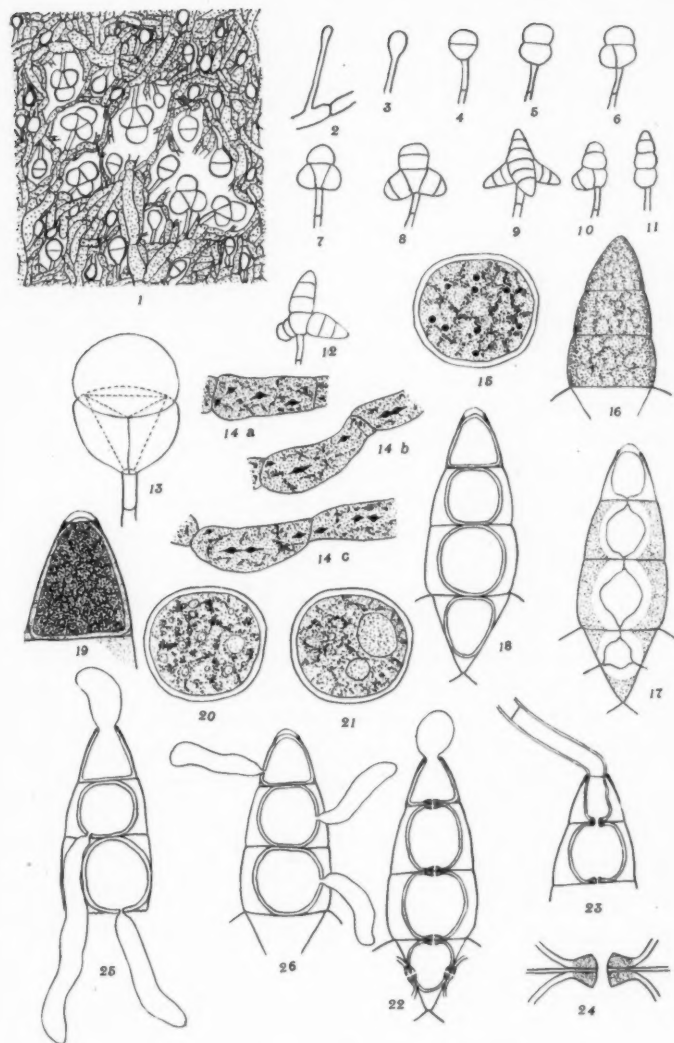
Fig. 22. Longitudinal section of germinating arm. The terminal cell has formed an irregular germ tube. Pores are evident between cells.

Fig. 23. The germ tube has elongated greatly and the terminal cell has drawn away from the exosporium. A cross wall has formed just within the germ pore. The germ tube has formed a heavy, peripheral wall and appears to be a continuation of the terminal cell. The cells are connected by canals of cytoplasm that extend through the cross wall, i.e., through openings in the thickened structures.

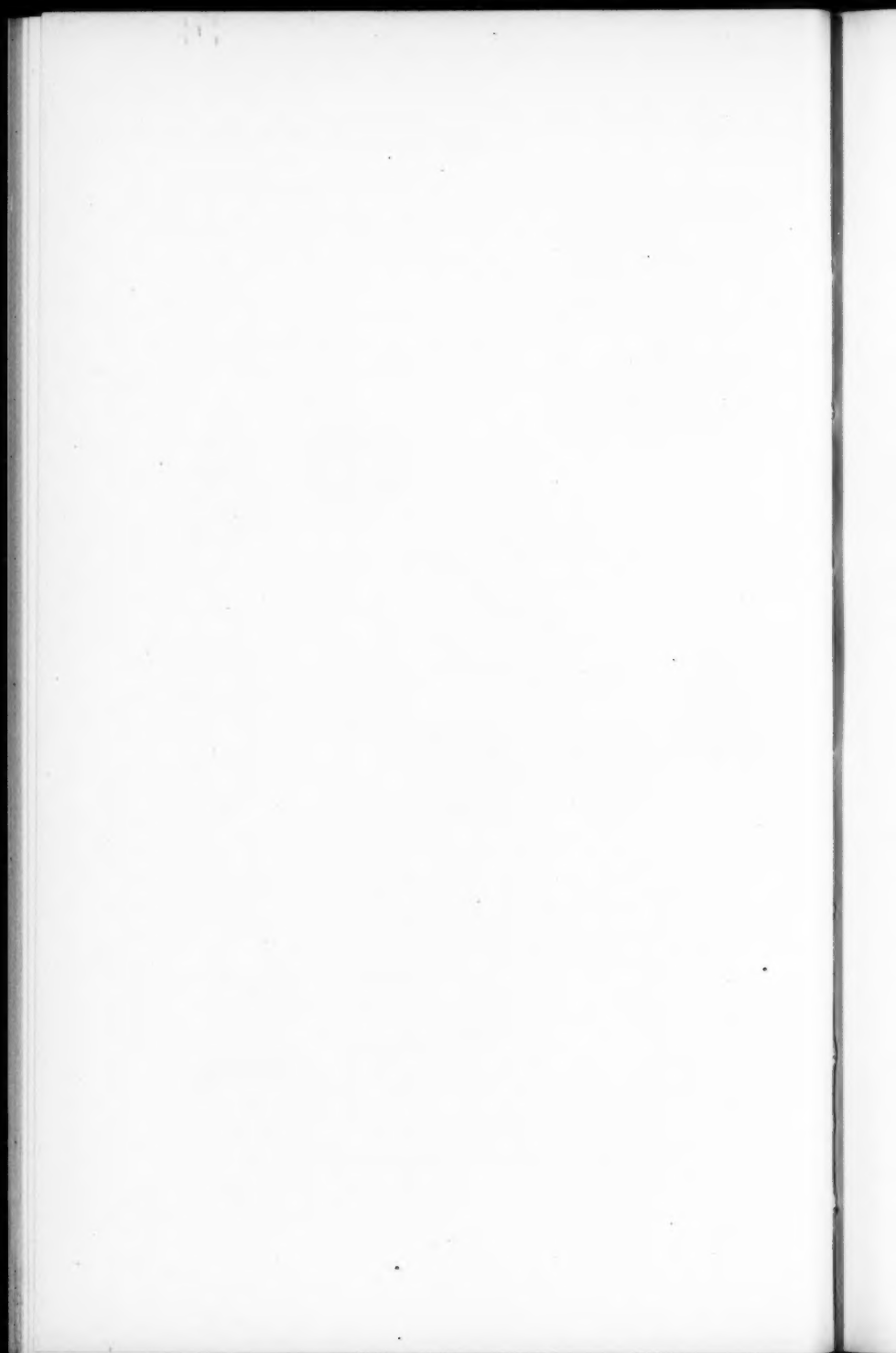
Fig. 24. Detailed, longitudinal section; diagram of the structure between cells. It consists of thickened tangent portions of the endosporium on either side of the cross wall, with an opening through the center.

Fig. 25. Fragment of crushed conidium. Germ tubes arise in the lower cells and grow out through the open, broken end; the terminal cell germinates normally.

Fig. 26. A typical germination of a conidium that has been manipulated does not germinate through the regular germ pore. The germ tubes arise laterally. The apical cell



ASTEROSPORIUM HOFFMANNI KUNZE



MYCOLOGICAL NOTES FOR 1921-22¹

L. O. OVERHOLTS

(WITH PLATE 17)

1. SEPTOBASIDIUM PINICOLA Snell.

This species was described by Snell in MYCOLOGIA 14: 55-59, 1922, and reported as occurring in New England, New York, and Idaho. I had noted it in abundance in New Hampshire during the summer of 1918 and in a list of species of fungi reported from that state in 1921 noted² its general characteristics, though through error the notes were run in under No. 74 of that list, to which it has of course no relation. My specimens were not fertile, and the statement was made that the plants had the appearance of a resupinate *Stereum*, but should have said *Thelephora* instead. Since that date I have observed it in several localities in central Pennsylvania, and I find in the Pennsylvania State College herbarium a collection made as early as 1914, at Charter Oak, Pa. Several other collections have been recently made at localities in the vicinity of State College. The fungus is always on the bark of *Pinus Strobus* and typically on young thrifty trees from ten to thirty years of age where they occur in a fairly dense association. The fungus is well illustrated and described by Snell.

2. BOLETUS PARASITICUS (Bull.) Fr.

In the North American Flora and in subsequent citations Merrill lists this species as "occasional in New England and New York." It was collected in Bear Meadows, Center Co., Pa., in

¹ These notes on new and little known fungi are a continuation of similar short papers begun in 1919 and published for that year in MYCOLOGIA 12: 135-142. 1920, and for the following year in Bull. Torrey Bot. Club 49: 163-173. 1922.

Contribution from the Department of Botany, The Pennsylvania State College, No. 46.

² Mycologia 13: 31. 1921.

the autumn of 1921, on its usual host, *Scleroderma*. By this habitat it is always easily recognized. (Plate 17, fig. 1.)

3. *HYPODERMA LINEARE* Peck

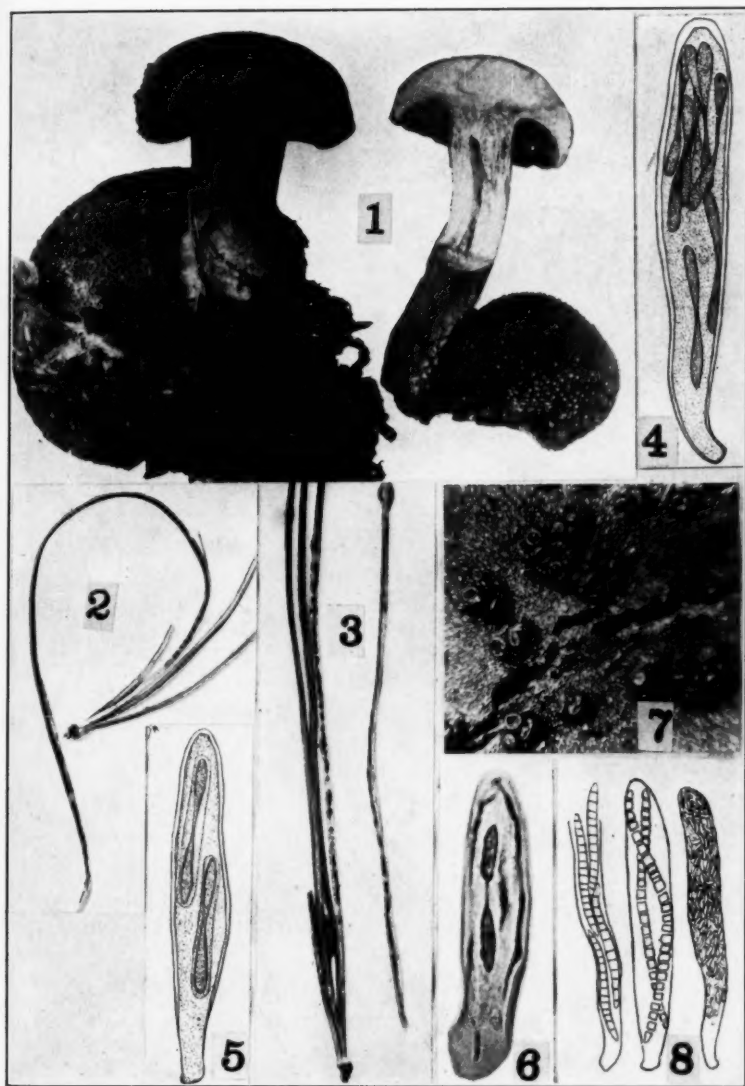
This species was originally described³ by Peck as a *Rhytisma* but was later (1878) transferred to the genus *Hypoderma*. Little reference to it is to be found in the later literature. In 1913 Graves⁴ noted a fungus on white pine in North Carolina that he thought might be referred here, but seemed to be at the same time only a variation of *H. brachysporum*. He was unable, however, to obtain the ascospores of the fungus.

The writer collected the species on leaves of *Pinus Strobus* in Stone Valley, Huntingdon Co., Pa., July 8, 1920 and again at Greenwood Furnace, Pa., Oct. 29, 1921. When examined microscopically the peculiar shape of the spores attracted attention and the specimen was referred to the above species. Later Dr. House kindly sent me fragments of the type collection at Albany and the reference was verified.

The plant may be said to have at least three characteristics in which it departs from the usual members of the genus. The apothecia produced on the needles are often elongated or linear in form, sometimes reaching a length of 5 mm. Many of them are much smaller, sometimes no more than half a millimeter in length, though many measure about 1 mm. Fertile fruiting bodies of the elongated type are not to be confused with a much more extensive blackening of the leaf that may occur in linear patches along the entire length of the leaf. These blackened areas, while apparently composed of fungous tissue, never develop asci and spores, and such sterile bodies are mentioned by Peck and by Graves, the former stating that "the specimens I have seen are seldom fertile," and again as forming "a thick black line on the lower surface of the leaf, often extending the entire length." The spores furnish a third character of diagnostic importance. They are surrounded by a wide mucous envelope or sheath, that dissolves away and disappears in KOH solution, and in addition are attenuated at the middle, the appearance being that of two large

³ Bull. Buf. Soc. Nat. Sci. 1: 71. 1873.

⁴ Phytopathology 3: 139. 1913.



1. BOLETUS PARASITICUS
2-6. HYPODERMA LINEARE
7-8. SCOLECONECTRIA SCOLECOSPORA

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cells connected end to end by a narrow neck, as pointed out in the original description by Peck. The body of the spore measures $57-70 \times 4.5-6 \mu$ as found in the type collection, though measurements of several spores in my material gave an average length of only $45-55 \mu$. Yet it is easily apparent under the microscope that Peck's material is much more mature than my own. I have not been able to distinguish a cross wall in the isthmus of the spores. These features are brought out in the accompanying illustrations (Figs. 2-6).

Little can be added concerning the parasitism of the species. In one of my collections the fungus was found only on the oldest remaining needles that would normally be shed at the end of that season. In the other case parasitism seemed to be more evident, though in no case were the leaves of the current season damaged. Those of the preceding year were heavily infected and had a grayish wilted appearance, while all leaves prior to those of that season had already disappeared.

4. HYMENOCOAETE AGGLUTINANS Ellis

This is a common species in the Appalachian mountain region, growing on a variety of living hosts. Dr. Burt mentions *Alnus*, *Benzoin*, and *Acer* as among its usual hosts. *Alnus* and *Betula* are the common hosts in this locality. One collection was made in Laurel Run, Center County, Pa., in 1921 cementing together twigs of *Rhododendron* and *Alnus*. Another collection was taken from *Viburnum dentatum*. On another occasion in 1922 it was found in Bear Meadows, Center Co., Pa., on the trunk of a living hemlock (*Tsuga canadensis*) sapling. The cementing habit mentioned by Dr. Burt as characteristic of the plant is not always present. At least I have found specimens on the trunk of an *Alnus* three and a half inches in diameter on which the fungus forms a large effused patch without evidence at present that its habit was ever a cementing one.

5. PHOMOPSIS JUNIPEROVORA Hahn

This destructive parasite of *Thuja* was found by the writer at State College, Pa., on both *T. occidentalis* and *T. orientalis* during

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the season of 1922. In both cases the trees were badly diseased and were dying at the time they were discovered, the upper two thirds of one being entirely dead.

6. WYNNEA AMERICANA Thaxter

A curious specimen of Discomycete was sent in September, 1922, by Rev. E. C. Smith of Meadville, Pa. Dr. Seaver to whom the specimen was referred by the writer determined it as *Wynnea americana*.

The species was published by Thaxter in the Botanical Gazette (39: 246) where that author discusses the other two known species of the genus, one from Mexico and the other from India. The present species was there reported as being found in Tennessee, North Carolina, and Ohio. Although this report is not very recent, probably few if any new localities have been reported for it since that date. Its occurrence in Pennsylvania seems therefore to be of considerable interest. Dr. Thaxter reports that the plant grows from a sclerotium but none were sent with the present specimen. The article previously cited contains an excellent illustration of the fungus. The longitudinal markings on the spores are only faintly visible in sections preserved in glycerine and where best developed give somewhat the appearance of the ridges and furrows on a butternut shell. One point not mentioned in Thaxter's diagnosis is that sections in 7 per cent KOH assume a vinaceous brown color.

7. SCOLECTECTRIA SCOLEOSPORA (Bref.) Seaver

Seaver in the North American Flora reports this species as occurring on various species of pines. I have so far collected it on *Pinus ponderosa*, *P. Strobus* and *P. sylvestris*. In addition, in April, 1922, it was found in Bear Meadows, Center Co., Pa., on a dead sapling of *Abies balsamea*. It was at first thought that the species might be *S. balsamea* but microscopic examination of the spores (Fig. 8) indicated rather *S. scoleospora*, and Dr. Seaver, to whom a part of the collection was sent, concurred in this opinion. So far as I have seen this is the first record of this species on other than a *Pinus* host.

The parasitism of this species is in need of investigation. The fungus is extremely common on *Pinus Strobus*, but according to the writer's observations is always found on trees that have been injured by other agencies. It is common on young trees of which the leader has been killed by the white pine weevil; it readily follows sun scald injury; and in regions infested with the white pine blister rust it occurs commonly in association with that disease. A certain degree of parasitism is indicated in some of these cases by the formation of definite and often pronounced constrictions developed far beyond that usually seen when these injuries occur alone.

8. *VOLUTELLA BUXI* (Corda) Berk.

In August, 1922, there were sent in from Bethlehem, Pa., specimens of *Buxus* on which the leaves and the smaller branches were dying. Definitely delimited spots were not formed on the leaves but they appeared to die from the tips back, about half the termi-

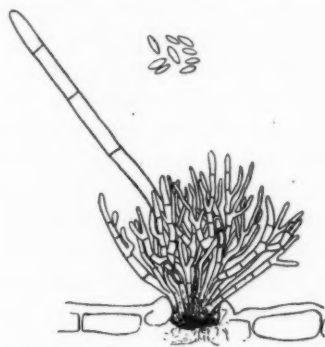


FIG. 1. Section through the fruiting body of *Volutella Buxi*, showing the conidiophores, a seta, and the spores.

nal part of each leaf being dead. In many cases the twig growth for the current year was entirely defoliated. On the lower surfaces of the dying leaves and on the defoliated twigs appeared small waxy, pink or rose-colored fruiting bodies, cushion-shaped or somewhat confluent. Sections through these bodies showed them to be characteristic *Volutella* stromata, with branched, septate

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conidiophores, cylindric septate setae projecting far beyond the stromata and 8–10 μ in diameter, and elongate-elliptic or narrowly fusoid, hyaline, one celled spores, 6–9 \times 2–2.5 μ . There seems little doubt that this fungus is properly referred to *V. Buxi* (Corda) Berk., although the setae are described in Rabenhorst Kryptogamenflora as 4 μ thick, and the spores as 10–11 \times 3.5–4.5 μ . More recently Moreau⁵ has noted the appearance of the fungus in France and describes the setae as 4–6 μ thick and the spores 10 \times 3.4 μ .

Moreau notes that the fungus is parasitic on the leaves, but in the collection cited above a *Phyllosticta* was also present although the two were not fruiting in abundance on the same leaves.

A more recent collection of this fungus was made in 1923 at State College by C. R. Orton.

9. PHYLLOSTICTA CONFERTISSIMA Ell. & Ev.

This species was originally described in 1893 from Louisville, Kansas, on *Ulmus fulva*, and Seaver in the North American Flora notes that it is known only from the type locality. A leaf-spotting *Phyllosticta* was collected by the writer on two occasions in the fall of 1922 at Charter Oak, Huntingdon Co., Pa. One collection was on *Ulmus americana* and the other was simply labeled as on *Ulmus*, and appears now to have been *U. fulva*. In this latter collection the spots formed are rather conspicuously delimited but on *U. americana* they appear more indefinite and of a dark rusty color. The very minute pycnidia are produced in great abundance on the lower side of these spots. The spores are allantoid, bacilli-form, 3 \times 1 μ . Specimens were sent to Dr. Seaver who verified the determination.

THE PENNSYLVANIA STATE COLLEGE,
STATE COLLEGE, PA.

EXPLANATION OF PLATE 17

Fig. 1. Sporophores of *Boletus parasiticus* attached to plants of *Scleroderma*. $\times 1$. Overholts Herb. No. 7764.

Fig. 2. Needles of *Pinus Strobus* bearing apothecia of *Hypoderma lincaire*. $\times 1\frac{1}{2}$. From the type collection in Herb. N. Y. State Museum.

⁵ Bull. Soc. Myc. Fr. 35: 12. 1919.

Fig. 3. *Hypoderma lineare* on *Pinus Strobis*. $\times 1\frac{1}{2}$. Overholts Herb. No. 7507.

Fig. 4. Mature ascus of *Hypoderma lineare* showing the eight characteristic spores. From the type collection. Drawn with camera lucida.

Fig. 5. Immature ascus of *H. lineare* showing two spores each surrounded by a mucous sheath. From the type collection. Drawn with camera lucida.

Fig. 6. Microphotograph of an ascus of *H. lineare* with a single spore. From the type collection.

Fig. 7. Perithecia of *Scoleconectria scolecospora* on *Pinus sylvestris* showing how they collapse at the apex when mature. Photographed with Planar lens. $\times 10$. Overholts Herb. No. 7985.

Fig. 8. Three asci showing the various types of ascospores produced. Drawn with the camera lucida. Overholts Herb. No. 7985.

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THREE NEW FUNGI

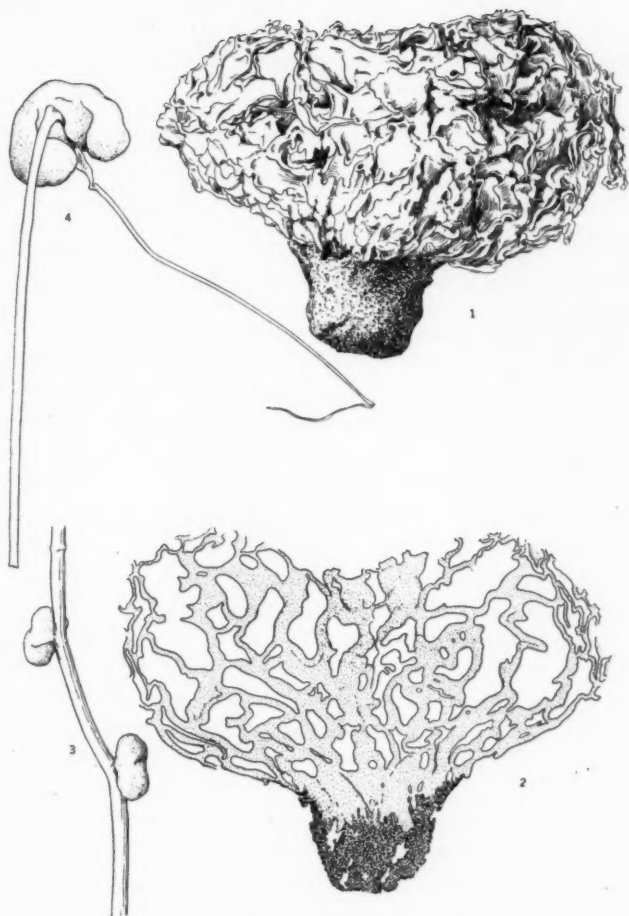
WILLIAM ALBERT SETCHELL

(WITH PLATES 18 AND 19)

Daleomyces gen. nov. Balsamiacearum

Ascomata magna, inferne per stipitem et contextam mycelialem curtam robustamque, truncato obconicam affixa, peridio maturitate proprio nullo vestita, interne labyrinthiformi-cavernosa; cameris numerosis cavis, clausis, forma magnitudineque irregularibus, earum parietibus hymenio vestitis; ascis elongatis, cylindraceis, octosporis, paraphysibus intermixtis; paraphysibus moderate robustis, ascos distincte superantibus, earum partibus exsertis flexis; sporis non septatis, oblongis, leviter ruguloso-reticulatis.

I am proposing a new genus of the Balsamiaceae in the sense of Fischer (in Engler und Prantl, Die natürl. Pfl.-fam., 1¹: 288) to receive a very interesting and peculiar hypogaeous fungus which has occurred several times in this vicinity. It is clearly a member of the Tuberales, even in the more limited sense in which the name is often used, and seemingly most nearly related to the genus *Balsamia*. In the adult stages, the only conditions as yet collected, it lacks a distinct peridium, has a definite region of attachment and has rough spores. In these respects, it differs materially from any species of *Balsamia* as yet described. From *Geopora*, it differs in having definite, closed and segregated chambers. It resembles, superficially at least, *Tirmannia* in its shape, definite organ of attachment, general direction of the trama walls, etc., but it possesses hollow chambers. There is something of a resemblance, also, to the more or less hypogaeous species of *Sepultaria* Massee, but that genus is truly a member of the Pezizales and may be looked upon as a sort of compound *Peziza* which has the additional peculiarity of being hypogaeous. We may think of the genus here proposed as a farther development of the *Sepultaria* type in much the same fashion as we may look upon *Geopora* as a more complicated development of a simple *Peziza* and upon



1-2. DALEOMYCES GARDNERI SETCHELL
3-4. THECAPHORA RUPPIAE SETCHELL

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Balsamia as the most complete development of the same type in the general direction of a typical member of the Tuberales.

The generic name is given in honor of Lawrence Dale Parks who discovered and kept under observation some of the best specimens obtained.

***Daleomyces Gardneri* sp. nov.**

Ascomata carnosa, alba ad dilute violascentia, late turbinata, 6-9 cm. alta, 9-12 cm. lata, exteriore profunde corrugata, inferne in stipitem latam affixantem abrupte attenuata; cameris formaque magnitudineque variantibus, cavis, earum parietibus hymeniis vestitis; ascis cylindraceutis, 220-250 μ altis, 8-12 μ latis, octosporis; paraphysibus ascos superantibus, moderate robustis, supra ascos lente tumidis et abrupte flexis, inferne rectis septatisque, 228-250 μ altis, 4 μ latis; ascosporis oblongo-ellipsoideis, 10-12 μ longis, 4-5 μ latis, leviter sed distincte ruguloso-reticulatis.

In earth (sandy), mixed with street sweepings, barely breaking through at the surface, Golden Gate Park, San Francisco, California, N. L. Gardner, No. 188, Feb. 29, 1904; in similar habitat, University of California Campus, Berkeley, California, L. Dale Parks and H. E. Parks, No. 1412, January 1923.

The plant described above is certainly puzzling in its general appearance and inclines one at first sight to refer it to the Pezizales, but its technical characters are clearly with the Tuberales. Its large size, fleshy consistency, whitish to pale violet or lavender color, its habit of showing itself only at maturity and then simply by slightly elevating and cracking the surface of the soil, all these contribute to its strange appearance. At Golden Gate Park, it was found in sandy soil richly fertilized with street sweepings. The locality in Berkeley showed a somewhat heavier soil and was situated where it received some street sweepings although much less than the locality in Golden Gate Park. It is hoped that more specimens may be discovered and in less mature stages.

***Acrospermum candidum* sp. nov.**

Perithecio e macula myceliali tenui, pallida areolataque oriendo, prope 1 mm. alto et 0.2-0.3 mm. in parte latissima crasso, superne candido inferne dilute melleo, breviter stipitato, supra stipitem moderate aut definite ampullaceo, superficie (supra stipitem)

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primo laxe squamato deinde regulariter areolato, haud compresso, apice orbiculate aperto, humido lente, sicco plane curvato; ascis dense stipatis paraphysibus intermixtis, longe cylindraceis, gracillimis, parallelis, octosporis ?, usque ad 500μ longis, circa 8μ latis; ascosporis elongatis, angustissime cylindraceis, hyalinis, septatis, circa 400μ longis et 1μ crassis, in ascis laxe spiraliter aggregatis; paraphysibus elongatis, gracillimis, lente flexuosis, circa 500μ longis et 1μ crassis.

On the undersides of living, but sterile leaves of *Alsophila quadripinnata* (Gmel.) C. Chr., collected at high levels, Cerro de Boqueron, State of Chiapas, Mexico, by Dr. C. A. Purpus (on No. 6715), Sept. 1913.

The species described above is closely related to *Acrospermum Maxoni* Farlow (cf. Riddle, MYCOLOGIA 12: 179, J1 1920), but differs from it in the color of the perithecium, in not being compressed and in its spirally arranged, septate spores. The scanty mycelium, superficial on the under surfaces of the pinnules of the host, shows in the earlier stages distinct hyphae whose walls seem to gelatinize so as to make the whole mass structureless at maturity. The mycelial fleck, at that time, becomes areolate sometimes showing the irregularities of the leaf surface. The short stipe is, at maturity, light honey colored and is in contrast with the shining white of the portions above it. The surface of the perithecium above the stipe disintegrates and peels off forming irregular scales. When the surface is finally denuded, it seems to have, when dry, an alveolate appearance. It is difficult to determine the exact number of spores and their length, but there seem to be eight, spirally twisted within the ascus, and, at maturity, distinctly septate. The figure of the ascus and its spores presented with this account is of a young ascus and is somewhat diagrammatic. In older asci, the spores are closer together, the spiral arrangement is more pronounced, the septation is more definite, but the course of the individual spore is difficult to follow. In the wet state, the perithecia are only slightly curved, but when dry, they are generally completely recurved. The finding of a second species of this genus on living ferns may indicate the possibility of there being even more of these interesting almost microscopic fungi to be looked for.

Thecaphora Ruppiae sp. nov.

Excrescentia irregulari tuberculoidea in caulibus foliisque *Ruppiae maritimae* usque ad 1 cm. diam. formans; sporis in intertextiis mediis excrescentium dense aggregatis, plerumque quaternis, sed aliquando binis, agglutinis, prope globosis, 4-6 μ crassis, parietibus crassis, levibus, fusciscentibus indutis.

While carrying on some cultures of *Ruppia maritima* var. *rostrata* Ag., my attention was attracted by some rather conspicuous galls forming here and there all along the stems and along the slender leaves. The *Ruppia* came, as seeds, from a brackish pool near the Power House at Alto in Marin County, California. At first, the galls were of a light green color and more or less translucent, but after a while, they changed to a dirty brown color. I have been able to obtain traces of the mycelium in the gelatinized condition. The spores are formed in dense layers surrounding the cells of the tissues of the galls intermediate between the central and the outer tissues. In slightly crushed material, the aggregations of spores seem to take the form of hollow spheres. This is due to their being formed around the rather large and swollen cells of the intermediate tissue layer of the gall. On farther crushing, the spores separate into the final aggregates which are largely in fours, but sometimes twos and uneven higher numbers are represented. The character of the spore aggregation places the species in the genus *Poikilosporium* of Dietel (cf. Flora 83: 87, 1897), but most writers seem inclined to unite that genus with *Thecaphora*. Unfortunately I have been unable to obtain the germination of the spores in my plants and the genus must, therefore, remain uncertain. It is of special interest to add a new species to the few known aquatic Ustilaginales.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CAL.

EXPLANATION OF PLATES

PLATE 18

Daleomyces Gardneri gen. et sp. nov.

Fig. 1. Ascoma in surface view, seen from the side. $\times 0.75$.

Fig. 2. Ascoma seen in median vertical section. $\times 0.75$.

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Thecaphora Ruppiae sp. nov.

Fig. 3. Galls on the stem of *Ruppia maritima* var. *rostrata* Ag. $\times 25$.

Fig. 4. Gall on the leaf of the same species. $\times 25$.

All figures drawn by Miss Anna Hamilton under the direction of W. A. Setchell.

PLATE 19

Daleomyces Gardneri gen. et sp. nov.

Fig. 5. Asci and paraphyses. $\times 165$.

Fig. 6. Ascospore. $\times 1300$.

Acrospermum candidum sp. nov.

Fig. 7. Pinnule of *Alsophila* seen from below and showing the perithecia in moist condition. $\times 1$.

Fig. 8. Similar pinnule showing the perithecia in dry condition. $\times 1$.

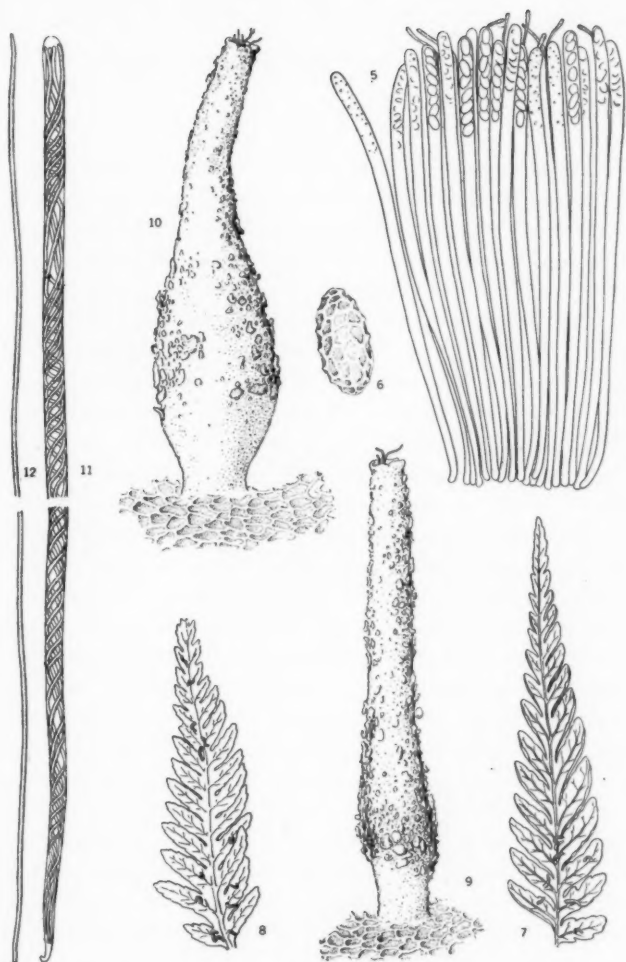
Fig. 9. Perithecium and portion of basal mycelium seen from the side. $\times 9$.

Fig. 10. Similar view of an older perithecium. $\times 9$.

Fig. 11. Ascus showing upper and lower portions. $\times 500$.

Fig. 12. Paraphysis showing upper and lower portions. $\times 500$.

All figures drawn by Miss Anna Hamilton under the direction of W. A. Setchell.



5-6. DALEOMYCES GARDNERI SETCHELL
7-12. ACROSPERMUM CANDIDUM SETCHELL

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FERN RUSTS AND THEIR AECIA ¹

J. C. ARTHUR

The rusts in general, a well circumscribed and readily recognized group of parasitic fungi, have not yet been assorted under any generally accepted classification, although the process of placing them in genera, families, orders, etc., has been actively proceeding for considerably over a hundred years. There are more than a thousand species of rusts in North America under about eighty genera. So great is the disparity in views between the most capable and advanced thinkers that there is no unanimity regarding what forms may be taken to represent primitive or ancient states, and what the most recent, specialized states. It is the situation the phanerogamic botanists were in before it was decided whether the cone-bearing plants (gymnosperms) were more suitably placed before all other flowering plants, in the middle, or at the end of the series. So long as the mycologists can not agree whether a short cycled form, like *Puccinia Xanthii* with its single stage and one kind of spores, is representative of rusts in their primitive condition in early geologic times, or a long cycled form, like *Puccinia graminis* with its several stages and diversity of spores, there is no hope for an acceptable classification of the rusts. If one were so blind as to be unable to tell which was the head and which the tail of a dog, there would be justifiable uncertainty as to the direction he would be likely to move.

It may be said that there are two classes of uredinologists, or students of the rusts. There are those who believe the rusts have progressed from a simple, one-spored condition in their early phylogenetic history, to the highly complex and heteroecious condition now known among many of them; and there are those who believe that the most ancient rusts had many kinds of spores and

¹ Presented before the Mycological Section of the Botanical Society of America at its Cincinnati meeting, December 28, 1923.

Contribution from the Botanical Department of the Purdue University Agricultural Experiment Station.

spore structures, and flourished upon a diversity of hosts, that by progressive development *pari passu* with the development of their hosts on the one hand, and the restrictive action of parasitism on the other, they have acquired great diversity of individual forms, but at the same time have become more and more specialized as to hosts and more and more reduced in their life cycles to fewer stages, in many cases even to a single spore stage without even the trace of a pycnium, the vestigial remains of a sexual structure, or even to the still greater simplification of a complete uninuclear, sexless condition. This problem of the progressive development of the rusts has nothing to do with their origin. It starts with the primitive rusts after they had acquired the characteristics of rusts with their parasitic habits, and loss of active sexuality. Their origin is another problem.

The problem in hand has been most commonly attacked from the morphological side, and this has been supplemented in late years especially by cytological studies. But there is another way of approaching the problem. The strict parasitism of the rusts has always been a dominant factor in their development, much as sexuality has been in higher plants. We should therefore expect that the rusts on the most ancient line of hosts would be likely to reveal more primitive features than those on hosts of later origin.

There is no question but that the ferns have the most ancient lineage of all the hosts of the rusts. If we study the fern rusts we ought to be able to get some clues to the character of the ancient forms. Most unfortunately there are only a few rusts known on ferns, and these have been the discoveries of recent years, although there is little doubt that many species exist and await the collector. Of those known only three kinds have had their life cycles fully traced out, and the imperfect state of our knowledge regarding the others must be pieced out by analogy.

The rust-bearing ferns belong to three families: the small family of the *Osmundaceae*, or cinnamon-ferns, much the oldest geologically, extending back into the Triassic and even the late Paleozoic and showing certain morphological resemblances to still older forms; the *Polypodiaceae*, containing the great majority of the ferns, but not so ancient, extending only to the Jurassic; and

the small family of the *Schizaeaceae*, or climbing ferns, geologically comparatively modern, although known in the Jurassic.

Now, to take up the rusts, let us begin with those found on the representatives of the most ancient known family of ferns, the *Osmundaceae*. Only one species occurs, *Uredinopsis Osmundae*. The genus *Uredinopsis* contains about ten intergrading species, which could with propriety be called varieties of one species, one form being on the genus *Osmunda*, and the other forms upon various genera of the *Polypodiaceae*. This is the first genus of fern rusts to be recognized, having been founded by Magnus of Germany in 1893, although some of the spore forms had received names under the genera *Protomyces* and *Septoria* as early as 1873, and *Gloeosporium* in 1880, clearly showing that for awhile they were not recognized as belonging to the rusts. The genus *Uredinopsis* is also the first of the fern rusts to have its life cycle fully worked out, which was done by Fraser (*MYCOLOGIA* 4: 189 and 5: 233) of Canada in 1912, who produced the white spored *Peridermium* on balsam fir (*Abies balsamea*) by placing the germinating teliospores of *Uredinopsis Osmundae* upon leaves of the fir. The characteristic pycnia and aecia appeared on the fir within two or three weeks. Four other species of the genus were similarly cultivated the same season, using partly teliospores and partly aeciospores for sowing. Let it be noted that the aecia inhabit a member of the *Abietineae*, one of the oldest families of coniferous plants.

What other evidence beside the association of hosts can be adduced that the rust on *Osmunda*, and similar forms, is representative of a primitive condition? In the first place all the spores and spore structures in the genus *Uredinopsis* are colorless; the characteristic yellow-brown coloring matter of rusts in general not having developed. Secondly, the teliospores are more or less scattered through the soft tissues of the host and do not arise from a well defined spore structure coming from a compacted primordium, as in most other rusts. In the third place, the urediniospores are markedly dimorphic, which does not occur among rusts outside of those on ferns. Both are characteristically conidial, and one of the forms is most unusual, reminding one of some of

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the ascomycetous fungi imperfecti, the spores being beaked and discharged as white vermiform threads. These spores are probably catenulate in origin, while the other form appears to be pedicellate and pulverulent, furthermore both forms have thin walls without evident germ-pores or the usual echinulation. It must be granted that these characters have a primitive aspect and are indicative of forms as ancestral and primitive as any we yet know among the rusts.

If we take *Uredinopsis Osmundae* as our best representative of the ancient rusts, let us name over the prominent features that an ancient rust, at least with this ancestry, must have had. To start with it was long cycled, having all the spore forms well displayed; pycnia, aecia, uredinia and telia. The pycnia were either subcuticular or slightly sunken beneath the epidermis, the aecia had spores in chains and a protective peridium, the uredinia had a less developed protective structure, with spores of two sorts, one apparently catenulate and the other pedicellate, both admirably designed for a conidial habit of repeating the generation, and the telia were thin walled and without the usual surface sculpturing. Finally the rust had the haploid and diploid generations well dissociated, one being developed on coniferous plants and the other on ferns.

Turning to the other fern rusts we find that *Hyalopsora*, a genus established by Magnus in 1901, having about the same number of species as *Uredinopsis*, but better differentiated, is developed entirely on the *Polypodiaceae*. The full life cycle for one of the species is known, and is likely to be typical of all others in the genus. Like *Uredinopsis* the rust is heteroecious, with aecia on *Abietineae*, being on firs (*Abies pectinata* in Europe and *A. balsamea* in America). The pycnia are subepidermal and well sunken in the tissues; the aecia like those of *Uredinopsis* have a well developed peridium; the uredinia are dimorphic, but not so strongly so as in *Uredinopsis* and with a less evident peridium; the urediniospores of both forms are apparently pedicellate and have a not unusual shape, with colorless walls without well formed echinulation, and with pores that can usually be detected; the teliospores are clustered in the epidermal cells. While all the

spores have rather thin colorless walls, the contents are often colored with the yellow pigment that belongs in the series of characteristic rust colors. Altogether the genus *Hyalopsora* is similar in its main features to *Uredinopsis*, but in all its stages shows an advance in the production of rust characters.

The third best known genus of fern rusts is *Milesia*, with about the same number of species as in each of the other two genera, all being on members of the *Polypodiaceae*. The aecia of one species are known, and occur on *Abies*. In all respects this genus shows some advance in characteristic rust features over *Hyalopsora*, and especially in having only one form of urediniospore.

A fourth genus, *Calidion*, also occurs on *Polypodiaceae*, but only two species are known and of these only the uredinial stage has been seen. The uredinia, however, are wholly unlike those of the other genera, for instead of being subepidermal, they are above the epidermis, surrounded by strongly incurved paraphyses, the whole sorus having been exerted through a stoma.

So much for what we may call one group of fern rusts, and the one containing the presumably most primitive known form on *Osmundaceae*. The *Schizaeaceae*, which harbors in large part the second group, may be considered a somewhat more modern family than the other two fern families, and it will not be surprising to find here a different style of rusts. Only two genera are known. One of these is a strikingly primitive genus, containing three or four species, one of the species being on *Polypodiaceae*, the others on *Schizaeaceae*. The teliospores for one of the species only have yet been found. The genus, *i.e.*, *Desmella*, is of recent discovery, being established by Sydow (Ann. Myc. 16: 242) in 1918. The whole life cycle is not yet made out, but one may surmise that it is heteroecious, and that the aecia occur upon angiospermous rather than upon coniferous hosts. The uredinia are noticeably echinulate, and are borne on pedicels that protrude through the stomata in tufts, but without paraphyses. The teliospores are in the same way borne on pedicels protruding through the stomata. The latter are smooth, two-celled by a transverse septum, and germinate without a resting stage. Both kinds of spores have somewhat colored walls and contents. One can not refrain from as-

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suming that here we have a very primitive form of the great group of *Puccinia*-like rusts.

The last rust on ferns to be mentioned is a species from Brazil on the climbing fern, *Lygodium*, a fern genus belonging to the *Schizaeaceae* of geologically recent origin. This species of rust is in every way similar to the usual conception of a *Puccinia*, and as it is probably heteroecious may be placed in the genus *Dicaeoma*. Both the uredinia and telia are in compact sori and subepidermal, aecia being unknown. The teliospores resemble those of *Desmella*, but are more strongly developed.

The evidence here brought forward, deduced from six genera and nearly forty species, seems strongly to support the view that the fern rusts taken together show primitive characteristics, and that primitive rusts, so far as we can judge of them from existing species, were long cycled, with many spore forms, and found their support upon two groups of unrelated hosts. How long such a condition existed before the full complement of spore forms and the saltation of hosts were reduced there is now no way of deciding, as fossil evidence is not available.

We may undertake to fit the six genera of fern rusts into series in which they will stand at the head of gradually expanding groups. It is easy to see that four of the genera of rusts on *Osmundaceae* and *Polypodiaceae* are to be taken as primitive forms of the family *Melampsoraceae*, and that the two remaining genera on *Schizaeaceae* and *Polypodiaceae* as primitive forms of the family *Pucciniaceae*. We may go farther and assume that *Uredinopsis* has been the precursor of *Pucciniastrum*, *Melampsorella*, etc., and that *Calidion*, as suggested by Dietel, is in line with the slightly known genus *Olivea*. It is even possible that *Desmella* is an early representative of *Hemileia*, or more likely of some other of the similar outlying *Puccinia*-like forms with superficial sori, and there can scarcely be a doubt that the fern *Dicaeoma* is an early representative of the numerous modern forms of *Dicaeoma*. But altogether too many gaps occur to enable us to go far in tracing a course of development that would string together any considerable number of the present rust genera. When aecia are discovered for the three imperfectly known genera of fern rusts, much more

light will doubtless be shed on the question of descent. It is enough for the present, if the evidence shows the most probable direction in which rust development has taken place, that is, from the complex to the simple, from long cycled kinds with spores of diverse forms to short cycled kinds with a single form of spore in addition to the basidiospore.

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NOTES AND BRIEF ARTICLES

(Unsigned notes are by the editor)

Dr. John T. Buchholz, professor of botany in the State University of Arkansas, spent a part of July and August at Cold Spring Harbor and the New York Botanical Garden in connection with his work on pollination and plant breeding.

Mr. Rafael Menendez Ramos, director of the experiment station at Rio Piedras, Porto Rico, recently spent a day at the Garden. He is especially concerned with the mosaic disease of sugar cane which is doing an increasing amount of damage in the sugar-growing sections of the West Indies.

Professor F. D. Kern of Pennsylvania State College and Professor H. H. Whetzel have recently returned from Porto Rico with an extensive collection of fungi for critical examination. They have been making a special study of the rusts of the island which group they are working in connection with the Flora of Porto Rico which is being published by the New York Academy of Sciences.

Under the title "*Petrakiella* Syd., eine neue Gattung aus der Verwandschaft der Diaporthen," a new species, *Petrakiella insignis*, is described and illustrated by H. Sydow (Ann. Myc. 22: 230-234) from material collected in southern Brazil by Theissen.

"Beitrag zur Kenntnis der Gattung *Cylindrosporium* Grev." (Ann. Myc. 22: 191-203, 1924) by von Hohnel contains notes on a number of American species and the new genera *Phloeosporella* (type *Cylindrosporium Ceanothi* Ellis & Ev.) and *Phloeosporina* (type *Cylindrosporium minor* Ellis & Kellerm.).

In "Mykologische Notizen" (Ann. Myc. 22: 1-182) Petrak has described the following new species and genera of fungi from America: *Amphisphaeria portoricensis* n. sp., *Pseudodimerium* n. gen., *Pseudodimerium meliolicolum* n. sp., *Thaxteriella* n. gen., *Thaxteriella corticola* n. sp., *Sphaerophoma* n. gen., *Sphaerophoma Brencklei* n. sp., *Davisiella* n. gen., based on *Cytodiplospora elymina* Davis, *Camarosporium asterinum* n. sp., *Hendersonia panicola* n. sp., *Clypeoporthella* n. gen., *Clypeoporthella Brencklei* n. sp., *Phomopsis Brencklei* n. sp., and *Shcaria* n. gen., based on *Camarosporium Magnoliae* Shear.

The frequent rains in May and June followed by warm weather toward the end of June brought out an unusually large crop of fleshy fungi on lawns and cultivated ground. *Naucoria semiorbicularis* was never more abundant. A specimen of *Stropharia melanosperma* was picked by me in front of the Museum Building on June 28 and the first plants of *Agaricus campester* were noticed the following day. In the woods, a yellow species of *Russula* was fairly common. Three weeks previous, I had collected *Cortinellus rutilans* at Hartsdale; but on June 29 in the Hemlock Grove I found the largest specimens I ever saw of this beautiful mushroom, which measured 15 cm. across and had a stipe 10 cm. in length and 2.5 to 3 cm. thick. Some distance away, I thought I had discovered *Melanolenca Russula*.

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